

论著

Prohibitin蛋白与卵巢癌紫杉醇耐药相关性的初步研究

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**摘要:** 目的: 研究转染pshRNA/prohibitin (PHB)的RNA干扰对卵巢癌紫杉醇耐药细胞株的生物学特性的影响. 方法: 采用Western印迹及实时PCR方法验证卵巢癌紫杉醇耐药细胞株(SKOV3/Taxol-25)和敏感细胞株(SKOV3)中PHB蛋白与mRNA的表达差异. 以脂质体Lipofectamine2000为载体, 将针对PHB基因设计的带有荧光蛋白的三靶特异性小发夹RNA干扰片段, 即pshRNA/PHB<sub>427</sub> (PHB1实验组)、pshRNA/PHB<sub>248</sub> (PHB2实验组)、pshRNA/PHB<sub>136</sub> (PHB3实验组)瞬时转染耐药细胞株, 另设阴性对照组. 转染48 h后, 用Western印迹及实时PCR方法检测PHB蛋白与mRNA的表达情况, 筛选出沉默效果明显的两个片段(PHB1及PHB3)为实验组, 与阴性对照组进行后续实验. 用MTT及流式细胞术检测实验组及对照组的细胞增殖、紫杉醇IC<sub>50</sub>及凋亡情况. 结果: 耐药细胞株中PHB蛋白相对表达量及mRNA相对表达量( $2^{-\Delta\Delta Ct}$ )明显高于敏感细胞株( $P < 0.05$ ). PHB1和PHB3实验组的蛋白相对表达量及mRNA相对表达量( $2^{-\Delta\Delta Ct}$ )较阴性对照组明显降低( $P < 0.05$ ). 转染48 h及72 h后PHB1及PHB3实验组细胞的增殖较阴性对照组明显减慢( $P < 0.05$ ); 干扰后72 h各实验组紫杉醇IC<sub>50</sub>较干扰前明显下降( $P < 0.05$ ); 转染48 h后PHB1及PHB3实验组细胞凋亡率较阴性对照组明显增加( $P < 0.05$ ). 结论: 针对PHB合成的shRNA能有效抑制耐药细胞株中PHB基因表达, 沉默PHB基因后耐药细胞株的细胞增殖能力下降, 凋亡率增加, 对紫杉醇敏感性增加, 提示PHB与卵巢癌紫杉醇耐药相关, 干扰PHB基因的表达可能降低卵巢癌紫杉醇耐药性.

**关键词:** RNA干扰 卵巢癌 prohibitin 紫杉醇耐药

Preliminary study of the prohibitin protein and paclitaxel resistance in ovarian cancer

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**Abstract:** Objective: To determine the effect of RNA interference with transferred pshRNA/PHB on the biological characteristics of paclitaxel-resistant ovarian cancer cell lines. Methods: Western blot and real time-PCR were used to assay the expression of PHB protein and mRNA in SKOV3/Taxol-25 and SKOV3 cell lines. The SKOV3/Taxol-25 cell lines were transiently transfected by 3 target-specific small hairpin RNA (shRNA) interference fragments with fluorescent protein named the pshRNA<sub>427</sub>/PHB1, pshRNA<sub>248</sub>/PHB2, and pshRNA<sub>136</sub>/PHB3. The empty plasmid transfection via vehicle Lipofectamine2000 served as a negative control. The expression levels of PHB protein and mRNA were detected by Western blot and real time-PCR after the transfection for 48 h. The silence effect of PHB1 and PHB3 groups was obvious. PHB1, PHB3, and the negative control groups were used for the following experiments. MTT and flow cytometry assay were used to test the cell proliferation, IC<sub>50</sub> of paclitaxel, and cell apoptosis in the 3 groups. Results: The expression levels of PHB protein and mRNA ( $2^{-\Delta\Delta Ct}$ ) were significantly higher in SKOV3/Taxol-25 cell line than those in SKOV3 cell line ( $P < 0.05$ ). The expression levels of PHB protein and mRNA were significantly lower in the PHB1 and PHB3 groups than those in the negative control group ( $P < 0.05$ ). The cell proliferations in the PHB1 and PHB3 groups were obviously slower than those in the negative control group after transfection for 48 h and 72 h ( $P < 0.05$ ). The IC<sub>50</sub> of paclitaxel in the PHB1 and PHB3 groups significantly decreased after transfection for 72 h compared with the negative control group ( $P < 0.05$ ). The cell apoptotic rate in the PHB1 and PHB3 groups significantly increased after transfection for 48 h compared with the negative control group ( $P < 0.05$ ). Conclusion: The shRNA/PHB can effectively suppress the expression of PHB gene in paclitaxel-resistant ovarian cancer cell lines. The cell proliferation in paclitaxel-resistant cell lines with removed PHB gene is significantly reduced. The apoptotic rate and the paclitaxel sensitivity of resistant cell lines with removed PHB gene are significantly increased. PHB gene is related to paclitaxel-resistance and interfering PHB gene expression may reduce paclitaxel resistance in ovarian cancer.

**Keywords:** RNA interference ovarian neoplasm prohibitin paclitaxel-resistance

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#### 参考文献:

- 1.Siegel R, Naishadham D, Jemal A, et al. Cancer statistics, 2012 [J]. CA Cancer J Clin, 2012, 62(1): 10-29.
- 2.Markman M, Hall J, Spitz DW, et al. Phase trial of weekly single-agent paclitaxel in platinum/paclitaxel-refractory ovarian cancer [J]. J Clin Oncol, 2002, 20(9): 2365-2369.
- 3.曹兰琴,黎欣,张怡,等. 卵巢癌紫杉醇耐药的蛋白质组学研究 [J]. 中南大学学报: 医学版, 2010, 35(4): 286-294.CAO Lanqin, LI Xin, ZHANG Yi, et al. Proteomic analysis of human ovarian cancer paclitaxel-resistant cell lines [J]. Journal of Central South University. Medical Science, 2010, 35(4): 286-294.
- 4.闫雪冬,李旻,张明伟,等. 不同方式诱导卵巢癌紫杉醇耐药细胞系的比较[J]. 基础医学与临床, 2006, 26(3): 284-289.YAN Xuedong, LI Min, ZHANG Mingwei, et al. Characterization of taxol resistance ovarian cancer cell lines induced by different administrations approaches [J]. Basic and Clinical Medicine, 2006, 26(3): 284-289.
- 5.马雪莲,查晓,张国楠. 卵巢癌紫杉醇耐药机制的研究进展 [J]. 肿瘤预防与治疗, 2008, 21(4): 436-439.MA Xuelian, CHA Xiao, ZHANG Guonan. Study of progress on paclitaxel-resistance of ovarian cancer [J]. Prevention and Treatment of Cancer, 2008, 21(4): 436-439.
- 6.Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly in vitro by taxol [J]. Nature, 1979, 277(5698): 665-667.
- 7.Theiss AL, Sitaraman SV. The role and therapeutic potential of prohibitin in disease [J]. Biochim Biophys Acta, 2011, 1813(6): 1137-1143.
- 8.Nijtmans LG, Artal SM, Grivell LA, et al. The mitochondrial PHB complex: roles in mitochondrial respiratory complex assembly, ageing and degenerative disease [J]. Cell Mol Life Sci, 2002, 59(1): 143-155.
- 9.Elbashir SM, Harborth J, Lendeckel W, et al. Duplexes of 21-Nucleotide RNAs mediate RNA interference in cultured mammalian cells [J]. Nature, 2001, 411 (6836): 494-498.
- 10.Audouy SA, de Leij LF, Hoekstra D, et al. In vivo characteristics of cationic liposomes as delivery vectors for gene therapy [J]. Pharm Res, 2002, 19(11): 1599-1605.
- 11.周雪雁,关伟军,马月辉,等. 脂质体介导转染法原理及其研究进展 [J]. 上海畜牧兽医通讯, 2004(4): 5-6.ZHOU Xueyan, GUAN Weijun, MA Yuehui, et al. The principle and progress of transfection conducted by liposome [J]. Shanghai Journal of Animal Husbandry and Veterinary Medicine, 2004(4): 5-6.
- 12.Back JW, Sanz MA, De Jong L, et al. A structure for the yeast prohibitin complex: Structure prediction and evidence from chemical crosslinking and mass spectrometry [J]. Protein Sci, 2002, 11(10): 2471-2478.
- 13.Arnold I, Langer T. Membrane protein degradation by AAA proteases in mitochondria [J]. Biochim Biophys Acta, 2002, 1592(1): 89-96.
- 14.Tatsuta T, Model K, Langer T. Formation of membrane-bound ring complexes by prohibitins in mitochondria [J]. Mol Biol Cell, 2005, 16(1): 248-259.

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1. 何艳,贺兴鄂,孙会卿,王文龙,雷建华.RNA干扰HBx基因对肝癌细胞化疗效果的影响[J]. 中南大学学报(医学版), 2009,34(05): 395-400
2. 任勇,任勇. RNA干扰抑制MDR1表达逆转Bel/7402/5-Fu肝癌细胞耐药性的研究[J]. 中南大学学报(医学版), 2006, 31(06): 872-876
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[J]. 中南大学学报(医学版), 2009,34(08): 723-730

4. 段绍斌,刘伏友,陈愷音,刘芳,李莹,凌光辉,肖力,刘虹,彭佑铭.转化生长因子 $\beta$ 1短发夹RNA对蛋白白致人肾小管上皮细胞细胞因子过表达的抑制作用[J]. 中南大学学报(医学版), 2009,34(10): 949-956
5. 刘惠宁1, 蔡净亭1, 林秋华2, 何可人1, 余蓉1.Caveolin-1与绒毛膜癌侵袭力之间的关系[J]. 中南大学学报(医学版), 2008,33(04): 331-337
6. 汤天凤1,周巧玲1,朱姗姗2,唐荣1,敖翔1.福辛普利、氯沙坦对肾小管上皮细胞TLR4表达的影响[J]. 中南大学学报(医学版), 2008,33(10): 958-965
7. 张芳,吴晓英.

[J]. 中南大学学报(医学版), 2006,31(01): 45-56

8. 张东山<sup>1,2</sup>, 刘伏友<sup>1</sup>, 彭佑铭<sup>1</sup>, 熊关钟<sup>2</sup>, 柴湘平<sup>2</sup>. 抑制Smad3的pSUPER RNAi系统的构建和活性鉴定[J]. 中南大学学报(医学版), 2007,32(06): 1042-1046

9. 杨晓苏, 胡益民, 肖波, 杨期东, 赵惠敏. 利用RNAi建立脊髓性肌萎缩症的细胞模型[J]. 中南大学学报(医学版), 2008,33(12): 1108-1112

10. 张勇, 杨欢, 肖波, 鲁特飞. RelB基因沉默的髓源树突状细胞负载Ta146~162

对TACHR预致敏T细胞免疫反应的影响[J]. 中南大学学报(医学版), 2010,35(1): 38-44

11. 曹兰琴<sup>1</sup>, 黎欣<sup>1</sup>, 张怡<sup>1</sup>, 彭芳<sup>2</sup>, 易红<sup>2</sup>, 许艳<sup>2</sup>, 李新国<sup>1</sup>, 王前<sup>1</sup>. 卵巢癌紫杉醇耐药的蛋白质组学研究[J]. 中南大学学报(医学版), 2010,35(4): 286-

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14. 刘虹<sup>1, 2</sup>, 刘伏友<sup>1, 2</sup>, \*, 彭佑铭<sup>1, 2</sup>, 袁芳<sup>1, 2</sup>, 刘映红<sup>1, 2</sup>. pcDU6质粒载体介导的TGFβ1 shRNA抑制TGFβ1在人腹膜间皮细胞中的表达[J]. 中南大学学报(医学版), 2004,29(5): 552-557

15. 谢贵元<sup>1,2</sup>, 胡春宏<sup>2</sup>, 黄明<sup>2</sup>. 上皮性卵巢癌RASSF1A基因甲基化与新辅助化疗疗效的相关性[J]. 中南大学学报(医学版), 2011,36(7): 631-633